Anopheles Mosquitoes

for Insectary Personnel

An introduction to cleanliness, biology, culture, genetics, and your productivity

Organized by ***Mark Q. Benedict*** of and for the CDC/ Division of Parasitic Disease, Entomology Branch
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Cleanliness and maintenance of the insectary and its contents

Introduction

"Nothing that is, came from that which was not."

When something hits the fan, somebody probably threw it.

You can't get something out of nothing.

Over a given period of log phase growth, the titer at the end point is proportional to the titer at the beginning.

This section covers practices that improve the quality of mosquito culture. It does not cover specific environmental conditions that are necessary such as temperature and humidity. These will be covered under 'Mosquito Biology and Culture.' Some of the sections are very specific, while others give principles to keep in mind when conducting the daily activities, planning procedures, and evaluating current ones.

Reasons to promote cleanliness and sterility

Reduce primary infections from pathogens

Many infections are fungal, protozoan, or bacterial and are either water or air-borne. While it is not practical to completely eliminate these, it is possible to reduce their prevalence in the insectary.

Reduce secondary infections

A primary infection may not be lethal or significantly debilitate the mosquitoes, but it may produce conditions that are favorable to the development of secondary pathogens that are lethal.

Reduce toxins released by fungi and microbes

Biogenic toxins can easily be reduced at the source; microbes.

Minimize pest attraction

Insect pests are of greatest concern in an insectary, although rodents might become a problem. Both of these can introduce waste products that harbor pathogens, or can carry them directly. (At one point in time, we had strong reason to believe that cockroaches consuming microsporidia-infected adults were dying in larval pans thus infecting another cohort.) Pests can be reduced by minimizing the conditions that attract them: food, accessible water, and harborages.

Control environmental and culture conditions

To the greatest extent possible, controlling the insectary conditions - both biological and physical - will promote robust, reproducible culture and experiments. An insectary filled with living organisms not deliberately cultured is a recipe for chaos.

Worker health and morale

A clean, pleasant-smelling, uncluttered insectary is both healthier and more desirable to work in for long periods. An uncomfortable and smelly insectary environment is one of the main reasons people are not eager to work in them. Moreover, an abundance of molds is likely to irritate asthmatics.

General methods to achieve partial and complete sterilization

(Purists will object that there is no such thing as partial sterilization. They are right. Something is either sterile or not. By partial sterilization, I mean a reduction in the number of microbes relative to the starting point.)

Heat including boiling, autoclaving, baking

Both fluids and containers can be exposed to different levels of heat. Even plastic containers that cannot be autoclaved can be partially sterilized by elevated temperatures. Liquids that contain components destroyed by autoclaving can be partially decontaminated by elevated heat.

Irradiation: gamma, X-ray, UV, photons, IR

Many types of irradiation can reduce the abundance of microbes. Although at first glance, the above might not appear to be appropriate for an insectary, they might be used in the ventilation system (UV), or for containers. Even sunshine will kill some microbes.

Chemicals including bleaching, gases, solvents

Bleach is commonly used to sterilize plastic containers, countertops, and floors. Ethanol also has some sterilization effect on bacteria and fungi, but be careful not to expose mosquitoes directly to ethanol since it will kill them instantly. Acetone is useful, although it too will kill mosquitoes and dissolves many plastics and floor finishes.

Desiccation

Extremely low humidity, especially in combination with elevated heat, reduces the abundance of many microbes. Therefore, plastic rearing containers dried and stored outside of the insectary are likely to harbor fewer microbes than those dried and stored inside the insectary.

Detergents

As a practical example, hand-washing with soap is more effective than using water alone since detergents break down cell membranes and kill microbes in the process. While excessive detergent residues will also kill mosquitoes, surfaces that are

cleaned with detergents will harbor fewer microbes. CAUTION: since detergents can kill or retard the growth of mosquitoes, rinse rearing containers thoroughly after detergent cleansing if it is used.

Cold including freezing and refrigeration

Unless special precautions are taken to protect the organisms, freezing will kill many microbes. Even those that will survive cold/freezing to some extent may be reduced in number or their growth-rate diminished.

Filtration

Ultra-filtration will remove fungi and bacteria from solutions. However, this method is usually only useful for small volumes of solutions due to the throughput and cost.

'Starvation'

Few microbes can survive indefinitely without minerals, complex organic matter etc. Cleanliness in general reduces 'food' sources.

Specific procedures to enhance sterility

Air and water

Properly maintained air filters

Filters are effective only if they are changed regularly. The demand and ultimately the performance of filters also depends on the cleanliness of the air entering the filter in the first place.

Properly maintained humidifiers

Most humidifiers contain a water reservoir that never empties completely. This means that even though deionized or even sterile water may enter the humidifier, airborne particles that fall into the reservoir will introduce sufficient material to establish microbial growth. These microbes will then conveniently ride on the water droplets into and onto everything they reach.

HEPA filters as a supplement

Recirculating filters utilizing activated charcoal, particulate meshes and HEPA are relatively inexpensive and readily available. Consider installation of these in addition to the filtration provided by the air-conditioning system.

Chlorinated, distilled, filtered or deionized water

Mosquito rearing water is a rich environment in which to grow microbes. Therefore, 'more microbes in, even more microbes out' is a good way to think of it. Use of any treated water will reduce microorganisms. However, some chemical treatment methods used in municipal supplies may not be compatible with larval mosquitoes. Test the source. Water from a hot water heater that is allowed to cool, or chlorinated water that is allowed to sit should be adequate. Chloramine is particularly of concern to invertebrates.

Larval food

Freeze powder

Keeping the powder frozen will not sterilize it, but it will prevent microbial growth and decay during storage.

Keep cold when in use

The growth rate of microbes is temperature-dependent. Keep the food in the refrigerator when mixed, and minimize the amount of time it is out on the bench. Mix only as much as you can use over a couple of days. Refrigerate the food overnight, and discard it if it's left out regardless of how 'good' it smells.

Clean container between batches

Soaking overnight in bleach is good. At a minimum, wash with a brush, a little detergent, and hot water. Rinse thoroughly in clean water.

Don't combine batches

e.g. You have 10 mls of mixed food remaining that you don't need immediately, but you will need much more later. You add the powder to the container containing the 10ml and add water. You've just inadvertently inoculated your fresh food with a nice starter culture of everything that will grow in it. Clean the containers between batches and don't mix them.

Adult sugar water

Clean hands

Think about what you transfer from cotton ball to cotton ball when you change sugar pads. "Did I just pick up a moldy cotton ball and stick my fingers in the fresh sugar water to get another?" Use one hand to remove the old cotton balls, the other for the fresh ones.

Clean cotton balls

Are the cotton balls sterile when I first open the package? They can be autoclaved in necessary. They can also be stored in sealed containers. An open bag of balls is a great settling ground for mold spores.

Clean covers / vials

Bleach screen covers and sugar vials. Store both in a closed container to prevent mold spores from accumulating on them

Sterile sugar water

Once a container of autoclaved sugar water is opened, it begins accumulating microbes. A cup of sugar water stored in the refrigerator and reused for weeks becomes increasingly contaminated over time. If you have a cup of cotton balls in sugar water, discard it weekly and start fresh.

Mosquito containers

Discard old mosquitoes from used containers quickly

Wet pans and cups are good places for microbes to increase. Dead mosquitoes in containers spew potential primary and secondary pathogens. Even if you autoclave materials, the dead mosquitoes and their microbes may have produced toxins before autoclaving or cleaning that still give you problems.

Remove dead mosquitoes from active rearing containers

If a pathogen killed a mosquito that is dead in the pan, you can be sure that when it decays it will release more into the water. Remove dead larvae and pupae when observed.

Autoclave as many types of containers as possible

This is the best way to eliminate microorganisms. Prefer use of autoclavable or disposable containers over reusable ones that cannot be sterilized effectively.

Dry thoroughly

Since desiccation kills many microbes, dry plastic containers and covers thoroughly before storage.

Consider bleaching

Bleach is a powerful oxidizer and the life of even plastics will be reduced if you use too much too often. However, for replaceable plastics, this is a good method to sterilize the surface. Be careful bleaching regular steel which rusts quickly when exposed.

Clean containers quickly after use

If you are unable to clean containers soon after using them, leave them stacked dry. Placing them in water for several days before cleaning is risky. If you do this, at least add detergent. The film of food, sugar water, dead adults etc. is great environment for growth of microorganisms.

Dry and store outside of insectary

The insectary is a convenient place to store supplies, but the high heat and humidity also make it a good place to grow microbes. Store as little there as possible.

Physical cleanliness

Walls

Wipe with detergent

Warm soapy water will do a lot to remove food sources for fungi, microbes, and kill them directly. Wiping the walls down occasionally to reduce mold growth also reduces the number of spores released into the air.

Keep walls accessible for cleaning

A wall behind a rearing rack is difficult to clean and unlikely to get cleaned. Keep a space between racks and the wall or use racks with casters that are easily moved.

Floors

Wipe up spills and eliminate leaks

Equip the insectary with a wet-dry vacuum cleaner. Make sure that the vacuum cleaner is not used elsewhere to vacuum toxins such as under furniture where insecticides have been sprayed. For all of the reasons above, and especially in relationship to desiccation, don't let water accumulate on floors, in containers, or on counters. Furthermore, the insectary structure and furniture will be a less favorable place for insects and microbes if it is sound and clean.

Mop with detergent

Again, remember that detergent may not be necessary to make the floor appear clean, but it does have an anti-microbial effect.

Shelves and counters

Remove unused equipment and stock

Unused materials in the insectary make it more difficult to clean. Moreover, the high heat and humidity tend to be hard on everything. If stock is stored in the insectary, it will begin to accumulate molds. Cardboard is especially nasty since it holds water, molds, and provides harborages for cockroaches.

Keep wiped and uncluttered

Shelves with nothing on them are easy to clean. Keep them uncluttered, dusted, and spill-free.

Remove dust

Dust is largely airborne and highly organic. It carries mold and bacterial spores and therefore circulation of dust by air in the lab spreads potential sources of infection.

Wipe counters

You are not only removing the spill. You are removing the spores of the organisms that grow in the spill, those carried by the pests attracted to the spill etc.

Storage containers

Use conveniently sealable containers

Choose storage containers that seal well, but are easy to seal since difficult lids are unlikely to be attached consistently. Tupperware-types are good and withstand bleaching, however you can't autoclave them.

Avoid cardboard, paper and wood. Use plastic, metal, glass

Paper products decay and host molds. The latter are more inert, less water absorbent, and (relatively) easily sterilized.

Stock items

Store the minimum possible in insectary

Move boxes of cotton balls, cups etc. into high heat and humidity areas when needed, but not as a storage solution.

Keep items sealed until use

Open one bag of cotton balls or one box of cups at a time.

Keep covered except when in use
Consider putting everything into covered containers such as plastic closet boxes or shoe-boxes.

Pest control

Regular preventative trapping

Ensure that the insectary is monitored for the presence of rodents, ants, and cockroaches. Ants particularly can clean out a cage of mosquitoes overnight, however cockroaches will also catch and consume living mosquitoes (Yes. We've seen this in insectary cages). Furthermore, both can spread microbes and leave feces in the insectary.

Reduce food sources

Spilled sugar water.
Lots of this gets used, and it's difficult to control. It helps though to use it over a counter-top rather than the floor and wipe it up daily.

Spilled food
Food is very protein- and sometime fat-rich. Ants and roaches love it, so keep the counters and floors clean.

Dead mosquitoes
You are what you eat. Since adult mosquitoes are also very protein and fat rich, they are good ant and roach food. Discard old cages ASAP.

Trash contents
Ensure that trashcans full of old cotton balls don't sit around the insectary for long periods. Custodians are generally responsible for this daily, so make sure they do their job.

Dirty containers
Dirty rearing pans are wonderfully moist and food-rich harborage for cockroaches. Clean them soon after use. Though cups are less accessible, lids are good places and the blood and frass is roach haute cuisine.

The mosquito life cycle and culture in the laboratory

Aspects of anopheline biology relevant to insectary operation

The following sections refer directly to the excellent book 'The Biology of Mosquitoes' Vol. I by A. N. Clements. This volume covers development, nutrition, and reproduction of mosquitoes and is published by Chapman and Hall, 1992. The second volume is also well worth owning.

Eggs

Fig. 3.3, p. 66 *Culex, Aedes*, and *Anopheles* eggs are laid in different patterns and this should be watched for as an indication of contamination.

Fig. 3.4, p. 68 *Anopheles* eggs are laid individually, have floats. Malformed eggs will sink and will seldom hatch.

Sect. 3.2, p. 69 Eggs are white when laid, darken and harden within a couple of hours.

**** Sect. 3.3.1 p. 70*** *Anopheles* eggs are not very resistant to drying and must be kept humid.

Larval Feeding

Sect. 4.1.1, p74 General components of larval environment and terms.

Table 4.1, p. 75 Gut contents of mosquito species vary.

Table 4.2, p. 77 Various species of mosquitoes feed by different modes and in different places.

Fig 4.1 and adjacent section on P. 78 Even within *Anopheles*, the specific mode of feeding varies.

Fig. 4.2, p. 80 Larval mouthparts are complex and suitable for both filter feeding and limited 'chewing.'

Sect. 4.3.2, p. 89 and Fig. 4.10 Anophelines create currents to direct food-particles to their mouths by using their mouth (palatal) brushes.

Sect. 4.3.3, p. 95 Due to the viscosity of the water and small particle size, collector-filterers do not actually filter! Particles are probably not filtered per se, but captured from the water.

Sect. 4.4.1, p 97 Feeding (in *Culex pipiens*) is stimulated by yeast extract (DOM) and nucleotides, however <u>particles alone were not adequate</u>

Sect 4.4.2, p 97 Particles ingested are small and increase in size with age.

Section 4.4.3, p. 98 Ingestion rates also increase as larvae mature...approximately 5X.

Growth and Development

Intrinsic effects on larval development

Sect. 7.1.1, p. 151 Males are generally faster-developing and smaller than females.

Extrinsic effects on larval development

Temperature

Sect. 7.1.2 and Figs. 7.1 and 7.2 pp. 152 and 153 Temperature is the most important extrinsic factor affecting growth rates of larvae.

Nutrition

Sect. 7.1.2, p. 154

Larval density and significant others?

Sect. 7.1.2, p. 154

Sect. 7.1.3, p. 155 Effects of larval 'health' on adults

Temperature

Nutrition and density (p. 157)

Salinity

'I've got rhythm. I've got music.'

Figure 7.10, p. 168 Effects of nutrition on larval-pupal ecdysis and periodicity.

Figure 7.12, p 169. Effects of light and temperature on adult emergence.

Adult feeding

Section 11.1.1, p 220. Plant juices are generally replaced by sucrose and fructose in the insectary.

Section 11.1.2, p 222. Blood is a source of protein for egg production.

Figure 11.1 Mosquito mouthparts

Figure 11.2 More female mouthparts

Figure 11.7 You're hooked. SEM image of maxilla of female.

Section 11.1.2, p 223. Diuresis allows females to increase protein content of blood meal.

Section 11.3.1, p 235. Males have 2 modes of feeding, but females have an additional one.

Section 11.3.3, p 236-237. 4 phases of blood-feeding

Exploration and probing (p 237)

Imbibing and withdrawal (p 239)

Manifestations of poor culture conditions

In the containers

Room-clearing malodorous water

Pan water should be fairly innocuous-smelling. It will never be so nice that you'll want to drink it, but it should not be foul.

Persistent bubbles on the surface of the water

When a pan of larvae is rocked sufficiently to create bubbles, observe them to see if they burst quickly. If they persist very long, excessive food / bacterioneuston / surfactant effect is scumming the surface of the water. Filtering the larvae or dragging a tissue over the surface may help in an emergency, but ultimately, the culture conditions should be changed. Active feeding reduces the formation of this if conditions are right since *Anopheles* surface feed (primarily), but also need to breathe through the surface as well.

Murky water

While the water will likely have a yellow color, particularly in the later stages, it should be fairly transparent except after feeding. You will develop judgement regarding how much opacity is appropriate. Greater opacity is acceptable in later stages, but should be avoided in the younger ones.

Irregular scum on the bottom of the container

It is normal that a thin film of organic matter accumulates on the bottom of the container. However, if it becomes thick, irregular in its reflectance, and contains bubbles, overfeeding is probably the cause. Change the container.

Stringy feces

If you observe unusually long fecal pellets, look for unusually clean water. This phenomenon occurs when larvae are starved. (I suspect that they are consuming their feces and they are more stringy the second time around.)

Extremely clear water

If the water has almost nothing suspended in it (except in the early stages), you're probably not feeding enough.

In the animals

Dead larvae

One rule of conversation: Never state the obvious. Less obvious is mortality in early stages. Young larvae decay quickly and are probably cannibalized. Therefore, it is not evident that they are missing at all. Only by counting hatch and / or young larvae can you determine survival rates.

Disparate larval stages are present.

Virtually all of the larvae should be at the fourth stage concurrently. If several instars are present at once, overcrowding / underfeeding are probably the cause.

Black spots internally or patches externally

Slow-growing larvae and those cultured in murky water often develop melanic nodules in the abdomen and thorax or black patches on the cuticle. These are both bad signs and the individuals that have these should be discarded. They seldom survive.

Fuzzy larvae

When food is overly abundant, Vorticella reproduce to such an extent that they cover the larvae and give them a fuzzy appearance (no kidding!). This is sometimes evident even without microscopic examination.

Missing and 'melanin' covered setae

When larval rearing conditions are sub-optimal, setae are often missing or covered with what is probably fungus. This can often be observed in the slowest-growing larvae even under good conditions, but if the condition is prevalent, it signifies poor general conditions.

Extended development time

At a constant temperature, the time from hatch to pupation should be predictable within a day one way or another. If pupation is delayed beyond a day or two of the expected time, several factors may be responsible: temperature is too low, inadequate food at some stage, excessive food in the early stages, wrong type of food.

Extended pupae posture

Pupae that are not curled into a comma shape but are extended with the posterior end projected horizontally *will not emerge*. You might as well discard these individuals. However, if you observe this among the first-forming pupae, it may not be too late to rescue the remaining larvae by changing the culture conditions.

Extended pupation time

Poor rearing results in disparate developmental rates and the ineluctable result that pupation is not complete even after several days. Ideally, but seldom achieved, is that all pupae to form in one day. Two days is great, three days typical.

Low frequency of emergence

The metamorphic transitions are the most sensitive stages to the effects of poor health. This can be observed as failure of pupae to emerge as adults. One should observe >99% of adults have emerged from pupa cups under good conditions.

Cessation of pupation

Once a larval population begin to pupate, they continue to do so until virtually all have metamorphosed. If pupation begins, but then ceases such that a smaller proportion of pupae are forming each day, poor culture is indicated. The problem is often underfeeding for a day or two.

Abnormally short adult survival

As in the above case, the effects of poor larval conditions are often evident as short-lived adults. Males especially - being the weaker sex - are sensitive to this effect.

Small egg batches per female

Studies have shown that above a weight threshold, the number of eggs laid per female is in fairly direct proportion to their adult weight. Since this is dependent on larval size, and that on larval culture, it is logical that larger larvae yield higher numbers of eggs per female.

Basic *Anopheles* Mendelian genetics or The Birds and the Bees…of *Anopheles*

Introduction

It is difficult to culture stocks on a long-term basis without some knowledge of their genetics. After all, what ultimately distinguishes stocks and species is their genetic constitution, not the name that we assign to them. The following are aspects of *Anopheles* genetics that are very relevant to understanding the day-to-day stability and integrity of stocks. To almost every generalization or definition that I provide, I can imagine an exception. However, the following will get you grounded in *Anopheles* genetics.

Following some concepts and terminology are simple problems.

The Facts of Life

Female Monogamy & Male Polygamy

To generalize the observations of numerous studies, *Anopheles* females mate only once in their life. The sperm with which the male inseminates the female are stored in spermathecae and are sufficient to fertilize several batches of eggs. Under typical insectary conditions, the sperm in the spermathecae are never depleted. Males, on the other hand, will mate several females if given the opportunity. One male can fertilize approx. 6-10 females. But again, males are seldom depleted of sperm in typical insectaries due to the limited number of virgin females - limited of course by the other males who are also trying to mate them. The genetic significance of these facts is that progeny from one female can almost always be considered as resulting from the mating of one male. The significance of *that* is that among the progeny from a single female (i.e. a 'family'), at most, four alleles will be observed.

Chromosome Number

All anophelines have a haploid number of 3: there are two autosomes (chromosomes not involved in sex determination) named chromosome 2 and 3, and one sex chromosome named X or Y. These are usually observed in mitotic spreads of brains and testes, however, salivary glands and/or ovaries may contain polytenized chromosomes in various species.

Sex determination

Sex determination appears superficially similar to humans and *Drosophila melanogaster*: females are XX and males are XY. The X chromosomes contain a region or regions of euchromatin, in which most expressed genes are located, and highly condensed heterochromatin which contains highly repetitive DNA and presumably few expressed genes. These regions are fairly distinguishable in chromosome preparations.

Genetic Glossary

Diploid

Having two sets of chromosomes.

Haploid

Having one set of chromosomes.

Gene

The basic unit of heritable expression. (There are other kinds of heritable expression, but gene covers 99.9% of them.)

Allele

Variant forms of genes

Locus

A general term for a place on the chromosomes. It may be a region, a base-pair, or functionally defined.

Linkage group

May temporarily be the genes or loci that are experimentally identified as not segregating independently from one another, but ultimately refers to the collection of everything located on one chromosome. The number of linkage groups identified experimentally may be greater than the actual number of chromosomes. (Think of contigs in a DNA sequence. One usually starts with several, but as the sequence in the gaps is obtained, multiple contigs coalesce into one.

Genotype

The allelic constitution of an individual, but sometimes applied to tissues or cells.

Phenotype

The *expressed* manifestation of a genotype.

Trait

Rough term meaning the same as a 'character'. Sounds more scientific than 'a thingie.' It is a functional description of some distinct behavior, form, color etc. Used the same as the way we would use it in common speech.

Homozygote

The condition in which a diploid individual has two apparently identical alleles of a gene.

Heterozygote

The condition in which a diploid individual has two different alleles of a gene.

Hemizygote

Usually refers to the state of having only one copy of a gene located on the X chromosome in males (which have only one X).

Homolog

One member of a pair of chromosomes.

Coupling

Also called 'cis.' Two alleles being referred to are on the *same* homolog.

Repulsion

Also called 'trans'. Two alleles being referred to are on the *opposite* homologs.

Pericentric inversion

A rearrangement of the chromosome in which a portion of a homolog is flipped and the centromere is included in the flipped region. Virtually all inversions of this sort in anophelines are induced by irradiation.

Paracentric inversion

A rearrangement of the chromosome in which a portion of a homolog is flipped and the centromere is NOT included in the flipped region. Important because often naturally occurring and useful as phylogenetic and population genetic tools.

Quantitative vs. discrete traits

Traits whose expression varies primarily in degree. For example: plant height, intensity of flower color, malaria parasite encapsulation. Discrete traits can be classified into classes e.g. *white eye vs. wild eye, collarless vs. collarless+, ebony vs. ebony+*.

Penetrance

A qualitative (low vs. high) or numerical value that refers to the Proportion of individuals in a population that express the phenotype that definitively identifies a particular genotype *when observed*. For example, in *A. gambiae*, c+ / c females have a red stripe on the larval dorsum *generally*. Something like 5% of c+ / c females do not appear to have a red stripe. So one could say that red stripe is about 95% penetrant.

Expressivity

The degree or Extent of expression of the phenotype. This addresses the issue not of what proportion of individuals have freckles, but how *many* or *how large* the freckles are. Or for anophelines, not do they have a red stripe, but how intense and clear is it.

Allelic relationships

Complete dominant

In a heterozygote, only the dominant allele is expressed, and the recessive allele is not. For example: c+ is completely dominant over c. *Stripe+* is dominant over *st* in *A. albimanus*.

Partially dominant or semi-dominant

In a heterozygote, the dominant allele is expressed to a lesser degree than in a homozyous dominant individual. For example: *ebony* heterozygotes are intermediate in darkness to either homozygote

Codominant

In a heterozygote, the phenotypes associated with both of two alleles present are observed. For example: microsatellite alleles are usually referred to as codominant markers because the repeat size of both alleles in a heterozygote can be observed. Another example would be enzyme electromorphs.

Epistasis

The phenotype of the expression of one gene eliminates the expression of another gene i.e. the phenotype of gene A prevents expression of gene B. *white* anophelines have no pigment associated with the *stripe* and *collarless* genes.

Gene interaction

The phenotype that is observed is different from that associated with gene A or gene B, but is a result of their combined effect. For example: *A. gambiae* that have *pink-eye* mutations generally have white or pink eyes. *A. gambiae* that have *red-eye* mutations have red eyes. When they have both *pink-eye* and *red-eye* mutations, the have 'pumpkin' colored eyes. Neither gene alone can produce this effect.

Effects of sex on expression

Sex-limited traits

Traits that are expressed in only one of the sexes. Most obvious are sexual morphologic characters like ovarian development. To use the same example again, why do only females express the red stripe since both males and females can have the c+ / c genotype? This observation is expressed by saying that red stripe is a sex-limited trait. May be determined by autosomal genes.

Sex-influenced traits

Traits that can be observed in both sexes, but the *kind or degree* of expression is influenced by the sex. For example: I lied for heuristic purposes. Sometimes red stripe *can* be observed in c+ / c males. However it is so faint relative to females that depending on where one draws the line between individuals that have a 'red stripe' and those that do not, you could say they don't show red stripe. Regardless, red stripe is so much fainter in males so that even if we do not consider it sex-limited, it is strongly sex-influenced. Traits of this class may also be determined by autosomal genes.

Sex-linked traits

Except for maleness, which is determined by the presence of a Y chromosome, sex-linked traits are determined by genes on the X chromosome.

Linkage Relationships

Autosomal linkage or inheritance

The Punnett Square to describe the pattern: An example using a *collarless* (chromosome 2) heterozygote mating. Fill in the geno- and phenotypes including sex.

		Female Gametes	
		c+	*c*
Male Gametes	*c*+		
	c		

Note that among the three genotypic classes of the progeny, **females and males occur at equal frequencies** in each.

Determine the genotypic frequencies

1. c+ / c _____
2. c+ / c+ _____
3. c / c _____

Determine the phenotypic frequencies

1. c+ _____
2. c _____

Sex linkage

The Punnett Square to illustrate the pattern: An example using *white* which is located on the X chromosome. Fill in the pheno- and genotypes including sex.

		Female Gametes	
		w+	***w***
Male Gametes	***w+***		
	Y		

Note that among the progeny, **females and males occur at different frequencies** in each phenotypic class.

Determine the genotypic frequencies

1. w+ / w female _____
2. w / w female _____
3. w+ / w+ female _____
4. w / Y (male) _____
5. w+ / Y (male) _____

Determine the phenotypic frequencies

1. w+ female _____
2. w female _____
3. w+ male _____
4. w male _____

Inheritance of two linked autosomal genes

The Punnett Square to describe the pattern: An example using *collarless* and *Dieldrin resistance* assuming 15% recombination.

		Female Gametes			
		Non-recombinant		Recombinant	
		Dl ^{R} *c+*	*Dl* ^{S} *c*	*Dl* ^{R} *c*	*Dl* ^{S} *c+*
Male Gametes	*Dl* ^{S} *c*				
	Dl ^{S} *c*				

Determine the genotypic frequencies

1. Dl ^{R} c+ / Dl ^{S} c _____ This is one 'parental' class
2. Dl ^{S} c / Dl ^{S} c _____ This is the other 'parental' class
3. Dl ^{R} c / Dl ^{S} c _____ This is one 'recombinant' class
4. Dl ^{S} c+ / Dl ^{S} c _____ This is the other 'recombinant' class

Determine the phenotypic frequencies

1. Dl ^{R} c+ _____ parental
2. Dl ^{S} c _____ parental
3. Dl ^{R} c _____ recombinant
4. Dl ^{S} c+ _____ recombinant

Basic laboratory stock population genetics

Introduction

Most stocks kept in insectaries are of value because of one or more unique alleles that are either fixed (pure-breeding) or polymorphic in the populations. On the other hand, some stocks consist only of 'wild type,' and no other stocks are present in the laboratory. In the latter case, the main concern is rarely contamination with a different species, but changes in allele frequency. In that case, the relative fitness of various alleles will determine whether one or another becomes extinct over time.

The following information is primarily directed toward the issues of maintaining alleles in polymorphic populations, and of the fate of alleles in contaminated stocks.

Hardy-Weinberg Equilibrium and the Binomial Equation

You're probably familiar with the assumptions of Hardy-Weinberg Equilibrium: a hypothetical model that predicts the frequency and stability of allele frequencies in populations given certain assumptions. According to their principles, the fundamental dynamics of the fate of alleles introduced into populations can be predicted by some fairly simple equations and applications of probability. While, these assume a few facts to be true, for Mendelian traits in laboratory stocks of mosquitoes, these approximate reality sufficiently that you can predict probable mating, allele, and geno- and phenotypic frequencies.

Two conclusions flow from their principle (quoted from *Genetics* by Strickberger, 1968):

"1. Under conditions of random mating in a large population where all genotypes are equally viable, gene frequencies of a particular generation depend upon the *gene* frequencies of the previous generation and not upon the *genotype* frequencies.

2. The frequencies of different genotypes produced through random mating depend only upon the gene frequencies."

The best-known method for determining allele and genotypic frequencies in a randomly mating population is a simple binomial equation (considering only two alleles):

$$p^2 + 2\,p\,q + q^2 \quad \underline{\text{which is the same as}} \quad (p + q)^2$$

The variables 'p' and 'q' are the frequencies of either of two alleles where p + q = 1. The frequency of the ' p p ' (homozygous) genotype is simply ' p^2 ' and the frequency of ' q q ' genotype is simply ' q^2 '. The frequency of the heterozygous genotype is ' 2 p q'.

Problem: *red eye* (*r*) is recessive to wild type (*r*+). You have a cage of randomly mating mosquitoes among which you observe 4 out of 100 *red eye* (*r r*) individuals.

If p = the allelic frequency of *r* and q = the allelic frequency of *r*+

p^2 =

p =

q =

Using the binomial equation, what are the expected frequencies of *r*+ *r*+ and *r*+ *r* genotype individuals?

Proportion *r*+ *r*+ (i.e. q^2) =

Proportion *r*+ *r* (i.e. 2pq) =

You have now estimated the frequencies of both the ***alleles*** and ***genotypes*** based on the observed number of *red eye* individuals.

Since we are assuming that individuals of all genotypes mate with one another randomly with no preference, we can estimate the probability of various *mating*s by simply multiplying the genotypic frequencies.

Problem: From the above cage of adults, you selected only wild-types and inbred them. You have individually egged 21 of these females and obtained 20 families of larvae. Among what proportion of families do you expect to observe *red-eye* individuals? (Use the Punnett square as an aid to calculate the expected mating frequencies. There is a little trick here. You have to *think*!)

		Proportion Females	
		Heterozygotes (*r*+ *r*) 2pq =	Homozygotes (*r*+ *r*+) q^2 =
Proportion Males	Heterozygotes (*r*+ *r*) 2pq =		
	Homozygotes (*r*+ *r*+) q^2 =		

In the above table, which matings will produce *red-eye* progeny?

If you isolated 21 females and obtained 20 families, approximately how many families will contain *red eye* progeny?

You may be wondering, "What is the probability that if I have 20 families I won't get *any* that have *red-eye* individuals just by chance?" Simple probability can be used to estimate the probability of getting various numbers of families like the ones above. (Usually, one is interested in knowing how many families you need to isolate to be pretty sure you'll get at least one like you desire; in this case a hetero X hetero mating.)

As a heuristic device to derive the formula, what is the probability of isolating one family and it does NOT have any *red-eye* individuals? It is simply…

1 - the probability that one DOES contain *red eye* which =

The probability of isolating 2 families, neither of which contain any *red eye* progeny is simply the probability of isolating the one above multiplied times the probability of isolating a second whose probability is EQUAL TO isolating the first family. (NOTE that these are independent events.)

$$p \times p = p^2$$

So if we take p to the 2^{nd} power for two families, we take it to the 'nth' power for 'n' families. So for 20 families, the probability of NOT seeing any families containing *red eye* is simply

$$p^{20}$$

Problem: *collarless* is a very common polymorphism in *Anopheles gambiae.* The *c* allele is recessive to *c*+ and therefore both ' *c*+ *c* ' and ' *c*+ *c*+ ' individuals have identical phenotypes. Suppose you wanted to purify a pure-breeding ' *c*+ *c*+ ' stock. You go to the G3 (an old wild stock from The Gambia) stock tray and out of 100 larvae, you find that 90 are phenotypically *c*+. You inbreed the *c*+ phenotype individuals. If you obtain 30 families, what is the probability that you will get at least one family that was the result of a mating between two ' *c*+ *c*+ ' individuals?

First, calculate the allele frequencies using the binomial equation.

If p = the allelic frequency of *c* and q = the allelic frequency of *c*+

p^2 =

p =

q =

Using the binomial equation, what are the expected frequencies of *c*+ *c*+ and *c*+ *c* genotype individuals?

Proportion *c*+ *c*+ (i.e. q^2) =

Proportion *c*+ *c* (i.e. 2pq) =

Remember, as in the above example, we're inbreeding ONLY the *c*+ phenotype individuals so you have to adjust the frequencies of the *c*+ types to reflect the fact that they now make up all (= 1) of the individuals being considered.

So you must adjust the proportions such that

Proportion *c*+ *c*+ (i.e. q^2) + Proportion *c*+ *c* (i.e. 2pq) = 1

		Proportion Females	
		Heterozygotes (*c*+ *c*) 2pq =	Homozygotes (*c*+ *c*+) q^2 =
Proportion Males	Heterozygotes (*c*+ *c*) 2pq =		
	Homozygotes (*c*+ *c*+) q^2 =		

We asked, "If you isolate 30 families, what is the probability that you will get at least one family that was the result of a mating between two ' *c*+ *c*+ ' individuals?" This is the exact same thing as asking, "If you isolate 30 families, what is the probability that ALL will be from matings between something other than *c*+ *c*+ types?"

$$p^{30} =$$

Fitness Effects in Polymorphic Populations

Finally, we consider polymorphic alleles in populations where the fitness of the various alleles is not equal. Fitness in the genetic sense refers simply to the relative reproductive success of an allele or a genotype. Mutant alleles are usually assumed to have reduced fitness relative to wild-types, but this is not always so. Maybe the *collarless* phenotype is less fit than wild-type. If so, what happens to the frequency of that phenotype and the *collarless* allele frequencies over time? Other alleles whose fitness we might want to consider in the insectary are recessive lethals, insecticide resistance alleles, and parasite susceptibility alleles.

To help understand the question, following are the fitness values (again from Strickberger) of several human genotypes. Note that some traits can have a *positive* fitness in certain environments.

Table 31–3

Relative fitnesses of certain human genotypes compared to normal homozygotes having a designated fitness of 1.00

Trait	Population	Relative fitness
retinoblastoma (heterozygotes)		0
infantile amaurotic idiocy (homozygote)		0
achondroplasia (heterozygotes)	Denmark	.196
hemophilia (males)	average of 3 European populations	.29
neurofibromatosis (heterozygotes)	Michigan	
	males	.413
	females	.748
Huntington's chorea (heterozygotes)	Michigan	
	males	.82
	females	1.25
sickle-cell anemia (heterozygotes)	East Africa (malarial regions)	1.26

From Spuhler, in Schull, 1963.

Fitness can be expressed as an adjustment variable 's' to the allele frequency. For example, a neutral allele with no effect on fitness has a relative fitness of ' s = 0 ' such that the allelic frequency adjustment would be q (1- s) or q (1 - 0) or simply q. On the other hand, a lethal has a fitness of 1 so that q (1- s) or q (1 - 1) becomes simply 0 (zero).

Obviously, how this is applied to the calculation of genotypic and phenotypic frequencies depends on the dominance relationship and whether the fitness effect is on zygotes or gametes, but suffice to say that generally the rate of effect on allele and phenotypic frequencies is proportional to the fitness. This is illustrated in the following table from Strickberger:

Table 31–6

Number of generations necessary for a given change in *q* of a deleterious recessive gene under different selection coefficients

Change in gene frequency		Change in frequency of homozygotes		No. generations for different s values						
From q_0	*To* q_n	*From* q_0^2	*To* q_n^2	s = 1 (*lethal*)	s = .80	s = .50	s = .20	s = .10	s = .01	s = .001
.99	.75	.980	.562	1	5	8	21	38	382	3,820
.75*	.50	.562	.250		2	3	9	18	176	1,765
.50	.25	.250	.062	2	4	6	15	31	310	3,099
.25	.10	.062	.010	6	9	14	35	71	710	7,099
.10	.01	.010	.0001	90	115	185	462	924	9,240	92,398
.01	.001	.0001	.000001	900	1,128	1,805	4,512	9,023	90,231	902,314
.001	.0001	.000001	.00000001	9,000	11,515	18,005	45,011	90,023	900,230	9,002,304

* The change in gene frequency is most rapid when $q_0 = .67$.

Several things should be observed from this table:

1. Observing the vertical axis trend, as the overall frequency of an allele decreases, the rate of decline in the allele (gene) frequency also decreases. Why? Since selection is occurring on homozygotes, the probability of individuals having two of the reduced fitness alleles becomes smaller as the probability of appropriate matings to produce these declines.

2. Observing the horizontal axis trend, as the fitness effect diminishes, the number of generations required for a given change in allele frequency to occur becomes greater. This is rather intuitive. We expect a lethal to have a more rapid effect on the frequency of the lethal allele than say an allele for laying an average of 110 vs. 111 eggs.

The take home messages of this discussion of fitness are the following:

1. You can expect lethals to be maintained in laboratory populations at low frequencies unless they are balanced by something or artificially selected.

2. Conversely, you can expect alleles with weak effects on fitness to be maintained at fairly high frequencies.

3. Genetic rearrangements such as inversions and translocations that cause semi-sterility and/or are homozygous lethal will decline in frequency and possibly become extinct unless deliberately selected for.

4. Recessive alleles that have reduced fitness relative to wild-type are very persistent even in the absence of selection.

5. Inbred populations tend toward fixation of the alleles with the strongest effects on fitness.

Efficiency and Productivity or Managing the Micro

Introduction

The goal for every employee should be to produce the maximum quantity of appropriate quality in as short a time as possible. This **is** productivity. Valuable employees consistently produce at a level above the minimum expected, and this performance usually **precedes** promotion to a level that pays commensurately.

In this section, we will discuss ways to increase productivity in repetitive tasks where sample confusion is likely. We will not say much about either **what** you should be doing or **why**. We begin with the assumption that you have been given a task, and it is up to you to perform it efficiently and reliably. The techniques used are common in laboratories that perform molecular biology and genetic work, and you can observe the highly productive people using them day-in and day-out. While laboratories that handle hundreds or thousands of samples are equipped with equipment and standardized procedures to keep samples straight and process them quickly, most small labs rely on the learned **work practices** of Individuals working in the lab for this.

Principles

Parkinson's Law: Work expands to fill the time available to perform it.

> "On days when I have lots to do, I get it all done, and on days when I don't have much to do, I only get that that little bit done, but I'm busy all the time."

This is a manifestation of Parkinson's Law. It results from a sub-conscious decision to slow the tempo of work so that the day is filled with activity. It is a primary identifier of so-called 'dead wood.' Set realistic goals ahead of time so that each day is filled with important activities, not merely those things that MUST be done. Continually working only to meet deadlines erodes time for adequate preparation, thinking, cleaning etc.

Focus on the product. Go 3D.

> "We're out there playing our hearts out every game. We're just not winning." -quote from '99 New York Mets player

Ask yourself, "What is the goal of the activity I'm performing right now?" If you cannot identify the activity as something that brings you closer to your goals, it's probably something that can be deferred, delegated, or deleted (3D). Clearly identify the *goal* and push aside extraneous matters ("playing your heart out") that do not serve that goal until it is accomplished.

(Classic example of wrong-headed thinking on this; giving 'Es' for effort. Which should be rewarded, working quickly and easily and getting the job done, or working hard and long and getting the job done.)

Don't think that the passage of time helps reach the goal

When building boats, I've often dreamed something like…

> "Next spring, I should have this sailboat completed and I'll be out there sailing. Wow!"

I found that as spring approached, the boat was not getting much closer to being completed. Why? I was 'thinking' that the passage of time brought me closer to my goal. Wasn't my goal going to be reached in spring? Well, spring is coming, isn't it? Bad logic.

Only the application of strenuous effort (*working* on the boat) brought me closer to my dream. Likewise, the passage of time *in and of itself* does not help you reach your goals.

Remedies

Activity Blocking

> "My insectary work takes forever some days. If I could just get in there and DO it, I'd be out in a couple of hours."

Have your materials ready when you start. Do you need to cut egging papers? Will you have to go to another room to get tape, cups etc? Each time you interrupt the flow of your work to go to another room and change activities, you are diminishing your productivity.

Once you start, stick to it. A trip to the bathroom or water cooler in the middle of an activity requires more time than a trip at the beginning or end. Why? Starting and stopping *themselves* eat up time. A task interrupted twice requires the time for 3 starts and 3 stops.

Don't be so nice. I love to help people solve problems. It's one of the most satisfying aspects of my job. However, interrupting an activity to help people will delay completion of mine. Offer to help them at a later time when you have completed your work. If they are able, ask them to solve their own problem e.g. "Could you call maintenance and ask them to unplug the drain?"

Schedule your work around natural breaks. Do you have 10 minutes before you plan to go out to lunch? Don't start a task that will take 2 hours, interrupt it, and come back later. Pick a quick 10 minute task (like cutting those egg papers), and come back to perform the task without interruption. You'll be more satisfied with the flow of your work as well.

Use carts and carrying trays. If you find yourself continually going back and forth from room-to-room for some piddly item, maybe you should purchase and use a piddly cart or piddly tray that goes with you. You'll be able to focus on the activity without running back and forth.

Schedule exceptional activities near the beginning or end of the day and natural breaks. If you have to walk to another buidling to check your mail, do it as you pass through at the beginning or end of the day on the way to your car, not in the middle of laboratory work.

Activity Layering

> "This restriction digest and cloning procedure takes so much time because I have lots of incubations when there's nothing to do."

Think ahead of time about the timing of the overall procedure. Think, "Ok, I'll need a gel poured to analyze the restriction fragments once the digest is done. I can pour that while the digest is going. But the PCR takes longer than the digests, so I'll start that first, start the digests, then pour the gel." Scheduling the events can turn a day's worth of activity into a morning's. This is especially important regarding Critical Path Activities (CPAs).

Identify Critical Path Activities

> "This project has taken so long to finish. It seems like it's crawling even though I'm being successful in the things I'm doing."

Identify the Critical Path Activities. These are events that will control, yes *control*, when your goal is reached. Failure to understand which activities of your project these are will delay the accomplishment. These activities have two important features:

1. They are essential links in a chain of events that are *predecessors* to other events called *successors*. For example: blood-feed females -> collect eggs -> hatch eggs -> culture larvae -> pick pupae -> sex pupae -> set up cross. Every event is a predecessor to the event that follows, and a successor to the one before. However, *they have a definite order*.

2. The time required cannot be minimized beyond a certain point e.g. the generation time of mosquitoes, the time required for a PCR reaction etc.

Specialize and set up an assembly line

If you are performing a repetitive activity that has many individual components, you are generally better off if you do ALL of one component at once, then do another all at once etc. For example, if your activity requires you to make 100 widgets, and each widget requires you to make and then assemble 3 parts; A, B, and C, you will perform the task fastest by making 100 part A, then making 100 part B, then making 100 part C, then assembling 100 widgets. A much slower alternative is making one A, making one B, making one C, then assembling the widget…100 times.

KIM at your service: Keep It Moving

> "It took me all month to get this procedure completed. I'm really frustrated."

Ask help from people coming in on weekends to do simple CPAs for you such as start cultures, perform transformations etc. This can accelerate the rate of accomplishing your task greatly.

Blood-feed something a little early rather than a little later.

Eliminate delays in critical path elements due to inattention and failure to plan. For example: you're cloning a restriction fragment that you need for experiments. Consider the following two ways of approaching the task.

Alternative A. On Friday, your supervisor comes up with an elegant and simple scheme to rearrange some plasmid parts to create a cure for all cancers…in mosquitoes. You spend Friday afternoon designing and discussing the procedure, and then you're on your own. You'll need to do a miniprep DNA purification from a fresh culture before you start. After a nice weekend, you start your overnight Monday around noon. On Tuesday morning, you help move the centrifuge. At 11:00, you start your DNA preps. After lunch, you set up your digests, but by the time they're finished, you're not sure you have time to run the fragment out before you leave and want to cut from a fresh gel. So Wednesday morning, you run the fragment out, have it gel-purified by noon, and ligate overnight. That evening you perform a transformation and have colonies Friday a.m. You set up 15 2-ml overnights to analyze by restriction digest on the following day (Saturday!). You purify plasmids from the 14 minipreps that grew, digest them, and run them out on the gel. Fortunately, you get the plasmid you want. You're frustrated because you missed having a day off.

Alternative B. On Friday, your supervisor comes up with an elegant and simple scheme to rearrange some plasmid parts to create a cure for all cancers…in mosquitoes. You spend Friday afternoon designing and discussing the procedure, and then you're on your own. You'll need to do a miniprep DNA purification from a fresh culture before you start. You request that a friend start the overnight on Sunday since they're coming in anyway. After a nice weekend in Chatanooga, you come in refreshed, but the centrifuge needs to be moved. You ask if it can wait until later in the day. It can, so you purify your plasmids and start the digest first thing. While it's incubating, you pour the gel. While the gel is running, you move the centrifuge, smash your fingers, and curse. Gel hasn't run far enough to separate the 14 kb fragment, so you go to lunch, gel purify the fragment by mid-afternoon, and set up the ligation overnight. Tuesday morning you perform a transformation. Wednesday a.m., you do colony pick PCR, and amplify the insert using standard primers and starting 2 ml overnights in the process. On the basis of the PCR, you identify 3 clones that appear to have insert. Thursday morning, you do alkaline lysis on two of the clones, digest them during lunch, and by the end of the day have confirmed the plasmids on a gel. Friday and Saturday, you return to Chattanooga since Sunday you're receiving the Nobel prize and want to be fresh.

Ask yourself two questions: "Which method requires more work?" and "Which method reduces the overall schedule time?

DO* it *FAST

If you have to walk from task to task, walk fast. It's healthier and saves time. Many activities are done just as well quickly as slowly.

Pay Attention to the Physical Layout

"I hate my bench when it's like this, all cluttered and messy. I just don't have time to clean it up, and it seems like I make more mistakes. I'm just too busy."

"Does anybody know what this rack of white tubes are with the numbers on them on my shelf in the minus 20?"

"I don't remember if I put enzyme in that tube or not. I think I did, but I can't afford to add more to make sure. I might already be at the maximum concentration."

"This is sooooo tiring labeling all these tubes. I spend half the morning just writing with this sucky Sharpie on these slippery little things."

"Occasionally I forget to put a label on one of the mosquito egging cups."

"How can I make sure I don't miss placing a fresh sugar vial in one of my cages?"

"I'm having a hard time telling if this tube is my PvuI or PvuII digest. The writing smeared."

Color code your tubes to identify digests, reactions, and steps in a protocol. This practice can save individual labeling.

Arrange the tubes in the rack and vials in the tray so that they are in the same order.

Fill the rack with tubes so that if one is missing and is not labeled, there is only one place it can go back into.

Stripe the tubes. If you have two sets of overnight cultures or DNA preps in a rack and both are labeled 1-10, draw a colored stripe or two across the top of all of them at once to distinguish clear tube #10 from clear tube #10 !

Label the bag or box…not the tubes. You've got 50 tubes of RNAase. Use colored tubes (if available) and place them all in a bag or box with a card indicating the color of tubes, concentration date etc.

Make two stacks: Before and After. Have I added a sugar vial? Have I egged them? Have I picked them? Make two clearly separated stacks between which you move things as the activity is performed. DO NOT move them back where they came from.

Make two rows: Before and After. Did I add the enzyme? Did I add DNA? No brainer.

Make a habit doing things in a certain order. That makes it more difficult to forget one.

Don't underestimate the importance of proximity. The time required for repetitive motions must be minimized or CPAs will be unnecessarily long. (See example on following page.)

Automate, but not automatically

> "This label maker is really cool. Now I can make specialized labels for all of my tubes. Wow!"

Though many things *seem* organized and *seem* like good ideas, an 'old fashioned' or 'compromised' approach may be adequate. For example, you may realize that even though the label maker is good, you have to turn it on, clear the previous label, change the tape, compose the label, print it, peel it, stick it. Does this really make sense for a set of miniprep DNAs that you'll screen and throw out in two days. A sharpie will do fine. Weight the cost vs. the benefit.

> "I have a database of all of my freezer stocks. I update it on the computer every time I add something. That way its' always current."

Again, a database for this is something I rely on. However, each time you add an item, if you do the above, you have to go to your computer, log onto the computer, open the database, make the change, save the database. Why not simply write the changes in your hard copy notebook in the lab, and when a few accumulate, do them all at once when you're at your computer. Sure, the database won't be current all the time, but is that really necessary? Use the hardcopy in your notebook where you make the record.

> "I want to use the mail-merge feature in MicroSoft Word to produce this form from a database."

How many forms do you need to make? Is it worth it to work out the mail-merge if you're only creating one form? It might be worth spending a whole week working out the details if you're making 500 forms, but if you can type one form in 10 minutes, it's not worth using mail-merge if it requires 15.

"Just DO it!"

Is it really necessary to *think* about doing this for so long? Sometimes the best thing you can do is to simply DO it. *Learn* not to ruminate, procrastinate, over-plan, over-think, and fiddle.

A final example demonstrates how performing the individual activities of a repetitive task even a little bit more quickly makes a large difference in the time required for the entire activity. In this case, observe how holding the pupa cup closer to the pipette reduces one part - hand motion – and makes a large difference in the total time required.

A slow & tedious task - picking pupae - made fast & tedious!

200 pupae and 200 larvae in a pan.

Picking 1 pan of pupae may be a critical path activity in your day.
Picking 1 pan consists of a series of sub-activities which are also critical path activities.

Get the pan out			Label cup			suck up pupa			hand motion			squirt pupa			
reps		time	reps		time	reps		time	reps		time	reps		time	
1	X	30	1	X	30	200	X	1	400	X	1	200	X	0.5	min
Total time		30			30			200			400			100	12.7

Get the pan out			Label cup			suck up pupa			hand motion			squirt pupa			
reps		time	reps		time	reps		time	reps		time	reps		time	
1	X	30	1	X	30	200	X	1	400	X	0.4	200	X	0.5	min
Total time		30			30			200			160			100	8.7